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Human herpesvirus 6 in the pathogenesis of multiple sclerosis

Review article

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Multiple sclerosis (MS) is one of the most common disabling neurological diseases affecting young adults. It is a chronic disease characterised by inflammation and demyelination. The aetiology of MS is still unknown, but involvement of viruses has been suspected for many years. Recently much interest has focused on human herpesvirus 6 (HHV-6), since the virus has been detected in MS plaques in the brain and patients with MS have been shown to have an aberrant immune response to HHV-6. Results from different studies are, however, conflicting and in the light of the long list of previous claims to have found the viral aetiology of MS it is necessary to interpret the HHV-6 findings with great caution. Possible mechanisms for virally induced demyelination and autoimmunity are discussed in this review, and the evidence for and against a role for HHV-6 in MS is summarised.

Key words: Multiple sclerosis; pathogenesis; human herpesvirus 6.

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MULTIPLE SCLEROSIS

The clinical picture of multiple sclerosis (MS) is extremely variable, reflecting the widespread affection of the central nervous system (CNS). The most common symptoms include impaired vision (caused by optic neuritis), sensory symptoms, motor symptoms, spasticity, ataxia, fatigue, incontinence, and cognitive impairment. The disease is usually divided into two types, relapsing-remitting MS and primary progressive MS. In most patients (85%), MS begins as relapsing-remitting MS with episodes of neurological symptoms followed by substantial or complete improvement. With time and repeated relapses, recovery is often less complete and a gradual relapse-independent clinical progression develops called secondary progressive MS. The age of onset of disease for relapsing-remitting MS is usually between 20 and 40 years. In primary progressive MS the decline in neurological function is gradual. This form of MS occurs particularly in patients in whom symptoms develop after the age of 40 years and primary progressive MS also has a slight male predominance while relapsing-remitting MS has a female:male ratio of 2:1.

PATHOLOGY

MS lesions are multifocal, and the distribution of lesions varies among different patients. However, there is a predilection for involvement of the optic nerve, periventricular white matter, brain stem and spinal cord (1). The reason for this is not completely understood, but might relate to a distinct microenvironment in these sites. For example, the proximity to the flow of cerebrospinal fluid and rich vascular perfusion might result in high concentrations of circulating inflammatory mediators, of other plasma proteins, or of blood-borne infectious agents. Alternatively, there is an as yet unknown differ-

ence in composition of myelin or extracellular matrix in these areas. Macroscopically, the MS plaques are well circumscribed and grossly visible and do not reflect any specific anatomic tract. The histological features of a typical MS lesion are infiltration of immune cells into the CNS and destruction of oligodendrocytes resulting in plaques of demyelination. The axons are relatively preserved, but axonal loss occurs and might be responsible for irreversible neurological disability in MS (2). An early event in all CNS immune reactions, and also in MS, is the enhanced expression of adhesion molecules on endothelial cells mediating extravasation of leukocytes. As immune cells migrate into the CNS, perivascular leakage occurs followed by complete disruption of microvascular wall integrity which permits inflow of plasma proteins into the brain parenchyma (1). The inflammatory cells found in the plaques are predominantly lymphocytes (mainly T-cells but also B-cells) and macrophages. The macrophages are foamy (lipid-rich) since they actively phagocytose myelin (3). In the initial phase of MS, oligodendrocytes are preserved in the lesions, immature oligodendrocytes proliferate, and remyelination can occur. In chronic plaques, however, the number of oligodendrocytes is decreased and remyelination is impaired (1).

EPIDEMIOLOGY

There is an uneven geographical distribution of MS with a much higher frequency in temperate zones compared to around the equator. This could mean that the environment is of importance for MS pathogenesis, but it might also reflect the distribution of genetically susceptible individuals. Migration studies have indicated that an environmental factor introduced to the individual at an early age is of importance for disease susceptibility, since children but not adults immigrating from a high risk to a low risk area (but not vice versa) acquire the risk of the new region (4). Great caution must, however, be exercised when interpreting results from migrations studies, since the migrants may not be representative of the population in their country of origin. Reports of clustering or "epidemics" of MS cases have also been used to suggest an important environmental influence, e.g. an infection. An example of this is the steep increase in MS incidence in the Faroe islands after World War I, where it has been argued that British soldiers stationed on the island introduced an "MS agent" to the population (5).

AETIOLOGY

The actiology of MS is still unknown, but it is generally presumed to be an autoimmune disease. It is likely that MS is caused by an interaction between genetic and environmental factors.

Genetics. There is increasing evidence that genetic factors have an important role in determining MS susceptibility. Large population-based studies of twins have shown a concordance rate of about 30% for monozygotic pairs and 2–3% for like-sex dizygotic pairs (6–8). These figures argue for two things: first, there is a substantial genetic component (which is probably polygenic); second, environmental factors are also of importance since the concordance rate is not 100% for monozygotic twins. Half-sibs have been shown to have the same risk of developing MS whether they are raised together or apart (9). This has been used as an argument against the importance of environmental factors in MS pathogenesis, but it might also reflect the role of a ubiquitous factor, such as a widespread infectious agent.

Immunology. MS patients show several aberrant immunological parameters. One important laboratory finding supporting the MS diagnosis is the local production of immunoglobulins in the CNS. This is seen on isoelectric focusing as oligoclonal bands present in cerebrospinal fluid (CSF), but not in serum from the same patient. The antigen specificities of the intrathecally produced immunoglobulins have been shown to be very varied. MS patients also have an increased immunological reactivity against several myelin antigens, including myelin basic protein (10), myelin-associated glycoprotein (11), and myelin oligodendrocyte glycoprotein (12, 13). There are furthermore signs of a defect in immunological suppression in MS patients, such as lower suppressor activity of CD8+ cells and decreased levels of different T-lymphocyte subsets (14–16).

Virology. Generations of researchers have considered whether a virus might cause MS,

and more than 20 different viruses have been suggested as the "MS agent" (Table 1). The speculation that there is a viral aetiology of MS is based on several observations:

- Epidemiological studies have indicated that an environmental factor, possibly an infection, encountered in childhood is of importance for disease susceptibility (5).
- A late encounter with common childhood infections such as measles, mumps, and rubella is more common in MS patients than in control subjects (17).
- Viral infections may trigger as many as 25% of MS exacerbations (18, 19).
- Intrathecal antibody production to several viruses is seen in MS patients.
- Other demyelinating diseases have definitely been shown to be caused by viruses, for example progressive multifocal leukoencephalopathy (JC-virus), postmeasles leukoencephalomyelitis, and HIV encephalopathy.

There are several theories as to how a virus could cause demyelination. The most obvious and direct mechanism is, of course, a lytic virus infection resulting in destruction of oligodendrocytes. Alternatively, expression of viral antigens on the surface of oligodendrocytes could lead to immune-mediated damage of the cells. Another concept is that of molecular mimicry, i.e. antigenic similarity between the microbe and human tissue triggering the immune system to damage normal tissue. Several viruses show various degrees of sequence homology with components of the myelin sheet, but still the existence of this mechanism of disease remains to be proven. Finally, a virally encoded superantigen inducing activation and uncontrolled proliferation of B- and T-lymphocyte clones has been suggested as a possible inducer of immune-mediated injury. In contrast, there are various scenarios where the detection of a virus in MS plaques is an epiphenomenon and not causally linked to the disease. A virus infecting leukocytes might, for example, be passively carried into the inflammatory lesion in the CNS. A latent virus infection might also be reactivated by the increased levels of cytokines and other inflammatory mediators in the MS plaques. An additive role of this epiphenomenon in the pathogenesis can, however, not be ruled out.

TABLE 1. *Microorganisms suggested in MS pathogenesis*

Microorganism	Reference
Rabies	(88)
Measles virus	(21)
Herpes simplex virus	(87, 89)
Scrapie agent	(90)
Multiple sclerosis-associated agent	(91)
Parainfluenza virus 1	(92)
Simian virus 5	(93)
Chimpanzee cytomegalovirus	(94)
SMON-like virus	(95)
Tick-borne encephalitis	(96)
Human T-lymphotropic virus I	(97)
Paramyxovirus SV5	(98)
Multiple sclerosis-associated retrovirus	(24, 99)
Human herpesvirus 6	(62)
Canine distemper virus	(100)
Epstein-Barr virus	(101)
Varicella-zoster virus	(102)
Chlamydia pneumoniae	(103)

MEASLES VIRUS

One of the most carefully studied viral agents in MS is measles virus. Acute measles causes immunosuppression, and one post-infectious complication is a demyelinating encephalomyelitis, raising the possibility that measles virus is involved in MS pathogenesis. The mechanism for measles virus-induced immunosuppression is not fully known. It has been shown, however, that measles virus infection downregulates IL-12, which is needed to generate cell-mediated immunity and drives the T-helper cell response towards T-helper 1 dominance (20). Since the original demonstration of higher antibody titres to measles virus in MS patients than in healthy controls (21), patients with MS have repeatedly been found to have elevated antibody titres to measles virus in both serum and CSF. Measles virus-like structures have also been seen by electron microscopy in MS brains (22). A late encounter with measles virus, but also other common infectious agents such as, for example, Epstein-Barr virus (EBV), has been correlated with an increased risk of developing MS (17). It has, however, not been possible to verify a role for measles virus in MS pathogenesis and the introduction of general vaccination pro-

grammes against measles has so far not decreased the MS incidence.

RETROVIRUSES

Since human retroviruses are in general known to impair the immune system and since the human T-lymphotropic virus type I (HTLV-I) causes a chronic, inflammatory, demyelinating disease (23), extensive investigations to search for a human retrovirus in MS patients have been performed. In 1989 a new human retrovirus called MS-associated retrovirus (MSRV) was found, first in CSF and later in monocytes from MS patients but not from control subjects (24, 25). Recently other groups have reported on detection of retroviruses in blood from MS patients (26, 27). There is also the possibility of an interaction between different viruses, for example that a herpesvirus could trigger retrovirus reactivation, as has been shown for HSV and MSRV, and suggested also for EBV (28, 29). Endogenous human retroviruses might come to provide new and interesting mechanisms for how autoimmune diseases are established, but such a link still remains to be proven.

HERPESVIRUSES

Another group of viruses that have been the focus of attention are the herpesvirus family. There are several features which make them attractive candidates: they are neurotropic, primary infection occurs early in life whereafter they remain latent and can be periodically reactivated, and they have the capacity to induce demyelination. HSV, varicella-zoster virus, and EBV have all been suggested to be involved in MS, but recently most interest has focused on human herpesvirus 6 (HHV-6).

HUMAN HERPESVIRUS 6

HHV-6 belongs to the Roseolovirus genus of the β-herpesvirus subfamily. HHV-6 infection occurs before the age of 3 in >90% of children, most frequently in the first year of life (30, 31). Horizontal spread through saliva is believed to be the most common route of transmission (32). On the basis of DNA restriction analysis, in vitro cellular tropism, and reactivities of monoclonal antibodies, two distinct variants of HHV-6 (HHV-6A and HHV-6B) can be separated. HHV-6 variant B is the aetiological agent of exanthema subitum (roseola infantum) (33), whereas HHV-6 variant A has not definitely been linked to any human disease. The virus propagates primarily in CD4+ T-lymphocytes, but infection can occur in a variety of human cells such as macrophages, dendritic cells, astrocytes, oligodendrocytes, and neurons (34–37). The cellular receptor for HHV-6 has recently been identified as CD46, which is present on all nucleated human cells examined to date (38).

HHV-6 affects several mediators of the immune system. It has been shown to induce apoptosis of CD4+ T-cells (39, 40) and CD8+ cells and natural killer cells are also usually killed when infected. HHV-6 inhibits IL-2 production and T-cell proliferation (41, 42). It induces tumour necrosis factor-α, IL-1β, and IL-10 (41, 43), which have stimulatory effects on the immune system. The HHV-6 ORF U12 has been found to encode for a functional β-chemokine receptor (44). β-chemokines are chemotactic cytokines that attract monocytes, eosinophils, basophils, and lymphocytes, but have no effect on neutrophils (45).

NEUROLOGICAL COMPLICATIONS OF HHV-6 INFECTION

The neuroinvasive potential of HHV-6 has been demonstrated in both immunocompetent and immunosuppressed individuals. Furthermore, HHV-6 has been associated with a wide array of neurological symptoms in these patients. In children with exanthema subitum, HHV-6 DNA can be detected in the CSF in between 23 and 70% of cases, indicating that viral neuroinvasion frequently occurs in primary infection (46, 47). The most common neurological complication of primary HHV-6 infection is seizures (31) and HHV-6 accounts for 26–31% of febrile seizures in young children (31, 48). It has been suggested that HHV-6 variant A would have a greater neurotropism compared to variant B since HHV-6A is found relatively more frequently in CSF (14%) than in peripheral blood

mononuclear cells (PBMC) (1%) in children with primary HHV-6 infecti n (49).

HHV-6 can cause meningitis and encephalitis in children and adults, as described in several case reports (50–57). In one retrospective study, HHV-6 DNA was found in CSF from 9 (7%) of 138 patients with clinical or laboratory evidence of encephalitis (58). Another study found intrathecal production of IgG or IgM to HHV-6 early antigen but not to 26 other viruses in 10 (20%) of 50 patients with meningitis or encephalitis, whereas none of 50 controls had intrathecal antibodies to HHV-6 (59).

Demyelination has also been associated with HHV-6, since the virus has been found in plaques of demyelination in two immunosuppressed individuals (51, 54) as well as in two immunocompetent young women with lethal encephalitis (60, 61). One of these had been clinically and histopathologically diagnosed as having acute MS.

HHV-6 AND MULTIPLE SCLEROSIS

In 1995, Challoner et al. demonstrated the presence of the gene for an HHV-6 DNA-binding protein (p41) in MS brains (62). Immunohistochemical staining revealed the presence of the HHV-6 proteins p41 and 101K in the nuclei of oligodendrocytes in MS plaques, while controls were negative or (rarely) contained HHV-6 antigen in the cytoplasm of oligodendrocytes. This finding focused much interest on the possible pathogenic role of HHV-6 in MS and has led to several studies on this subject.

Patients with MS have been found to have higher antibody titres to HHV-6 in both serum and CSF as compared to control patients (63–65). IgM antibodies to HHV-6 have also been reported to be more prevalent in MS patients (63, 66, 67). PCR studies have found HHV-6 DNA in CSF at higher frequency among MS patients compared to controls (65, 68). However, several studies of humoral response (69–71) as well as of DNA detection in CSF (72–74) have not verified these findings. HHV-6 DNA has been detected in serum or cell-free plasma from MS patients but not from controls, suggesting a more active infection in MS patients. On the other hand, detection of only latent HHV-6 genomes in PBMC from MS patients and rare detection of HHV-6 DNA in leukocytes from patients with relapsing-remitting MS argues against this (75, 76).

As mentioned previously, HHV-6 variant A has been suggested to be more neurotropic than variant B, since variant A was found to persist in CSF while variant B persisted in PBMC (49). Furthermore, the HHV-6A variant has been demonstrated in areas of demyelination in the CNS of AIDS patients (77). These findings point to the possibility of a role for the HHV-6A variant rather than the HHV-6B variant in MS, and there are reports supporting this. One study detected exclusively HHV-6A DNA sequences in PBMC from MS patients, while none of the controls had any detectable HHV-6 DNA in PBMC (78). Another study analysed the cellular immune response to HHV-6 in patients with MS, showing an increased lymphoproliferative response to HHV-6 variant A compared to controls (79).

In Table 2, data from several studies on HHV-6 and MS are summarised. As described above, the results from MS patients are conflicting. It is, however, important to note that there are large variations between the control groups used in different studies. For example, the frequency of detectable HHV-6 DNA in PBMC ranges from zero to 75%, and the frequency of HHV-6 IgM ranges from 2.5 to 18% between different control groups. This makes it very difficult to interpret and compare results from different studies, and standardisation of methodology and patient selection should be a priority in the future. Exchange of samples between laboratories could be a first step in investigating whether the discrepant results obtained in different studies are due to the different laboratory assays used, or reflect a true difference between the patient groups studied.

CD46

The human cell surface protein CD46 was recently identified as the cellular receptor for HHV-6 (38). Interestingly, this molecule also works as the receptor for the Edmonston strain of measles virus (80, 81), which has been suggested to be a possible MS-associated agent. CD46 is a regulator of complement activation, protecting autologous cells from complement-

TABLE 2. *Summary of results from different studies on detection of HHV-6 DNA and IgM to HHV-6 in MS patients and controls*

HHV-6 DNA						HHV-6 IgM		Reference
Cerebrospinal fluid		PBMC		Serum		Serum		
MS patients	Controls	MS patients	Controls	MS patients	Controls	MS patients	Controls	
				15/50 (30%)	0/47	14/22 (64%)[a] 6/14 (43%)[a]	3/35 (8.6%)	(67)
		18/24 (75%)	12/20 (60%)			15/21 (71%)	7/40 (18%)	(104)
						9/16 (56%)	5/29 (17%)	(63)
2/12 (17%)	0/4					1/55 (1.8%)	4/162 (2.5%)	(70)
3/51 (5.9%)	1/17 (5.9%)							(105)
		26/34 (76%)[b]	15/20 (75%)[c]	8/34 (23%)[d]	0/19			(106)
				2/32 (6.3%)	1/34 (2.9%)			(73)
		3/56 (5.4%)	0/20					(65)
3/21 (14%)	0/26			0/21	0/26			(78)
		7/34 (21%)[e]	0/20					(107)
				1/24 (4.2%)	0/30			(74)
0/6	0/14			0/32	0/12			
0/32	0/12							(66)
						21/25 (80%)	5/33 (15%)	(64)
		1/31 (3.2%)	1/24 (4.2%)					
		2/14 (14%)	0/9			0/25	0/9	(108)
0/25	0/9	8/20 (40%)	11/30 (37%)					(109)

[a] RRMS/CPMS, respectively.
[b] 87% HHV-6 variant A.
[c] 100% HHV-6 variant B.
[d] 80% HHV-6 variant A.
[e] 100% HHV-6 variant A.

mediated lysis, and is expressed n all human nucleated cells. There are different isoforms of CD46 and expression is dependent on the genetic background of the individual and on tissue-specific splicing (82, 83). It has been demonstrated that cells expressing CD46 mutants that lack a cytoplasmic part of the molecule are highly susceptible to measles virus infection and have a decreased ability to produce antiviral substances such as IFN-α/β and nitric oxide (84). This makes it tempting to speculate that individuals expressing a certain form of CD46 in the brain may respond inadequately to different infectious agent using this receptor, and that a receptor variant is a common finding in MS patients rather than one specific infectious agent. It has recently been demonstrated that clinical isolates of measles virus use SLAM (signalling lymphocytic activation molecule) rather than CD46 as a cellular receptor (85), but still the experimental evidence from the Edmonston strain can be used to speculate as to the mechanisms of virally induced disease.

Compared with previous evidence of a viral aetiology of MS, HHV-6 is of special interest since virally encoded proteins have indeed been found in oligodendrocytes in MS lesions. It is also known that HHV-6 is neuroinvasive and has the capacity to induce demyelination. Still the data on a possible link between HHV-6 and MS are conflicting, and most studies provide only indirect evidence by analysing antibody response or DNA detection for a ubiquitous virus. A way to prove more directly that a virus is important in MS pathogenesis could be to show a beneficial effect of antiviral treatment on the disease progress. One trial where MS patients were given antiviral drugs has been performed (86). This study was based on the hypothesis that herpes simplex virus is involved in MS, which was formulated upon the isolation of herpes simplex virus from CSF of a woman during her first attack of MS (87). A total of 60 MS patients with relapsing-remitting disease were followed for 2 years, 30 receiving 800 mg aciclovir daily and 30 receiving placebo in a double-blinded fashion. During this time the aciclovir group experienced 34% fewer exacerbations than the placebo group, but this was only a borderline significant difference ($p = 0.08$). Aciclovir is an antiviral compound with a documented effect on CNS infection with herpes simplex virus, but it has only a limited effect on β- and γ-herpesviruses, and the study is thus not very relevant for HHV-6. In order to target, for example, HHV-6 or EBV, it would be preferable to evaluate the two antiviral substances ganciclovir and foscarnet in a similar study.

MS is a heterogeneous disease, and the pathogenic mechanism is likely to be complex. Both autoimmune and virus-induced damage must be considered, and different viruses might even be associated with the disease in different individuals or different forms of the disease. Today it cannot be definitively concluded that HHV-6 is involved in MS pathogenesis.

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